

Isolation and Screening of Dye Decolorizing Bacterial Isolates from Contaminated Sites

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Abstract

Forty five bacterial strains were isolated from contaminated textile wastewater and soil, and then isolates were screened for their ability to decolorize textile dyes from aqueous solution. Initially twenty four bacterial isolates were screened based on their ability to decolorize a wide spectrum of dyes efficiently such as Black WNN, Blue FNR, Red FN2BL, Blue RC, TURQ Blue and Diresul RDT Black dye, by a rapid microtiter plate screening method. Among all isolates, NF-23 was found to decolorize maximum number of dyes followed by NF-22 and NF-21. NF-23 decolorized Black WNN (95%), Blue FNR (50%), Diresul RDT black (90%) and Red FN2BL (80%) after 72 h of incubation at pH-9 and temperature 35°C under anoxic condition. These results signify that bacterial isolates could effectively be used in development of alternative and eco-friendly method for decolorization and biodegradation of textile dyes from industrial effluent.

Keywords

Biodegradation; Decolorization; Microtiter Plate; Reactive Dyes

Introduction

Dyes are an important class of synthetic organic compounds, widely used in textile, leather, plastic, cosmetic and food industries and are therefore common industrial pollutants. Textile effluent released from industries is a complex mixture of many polluting substances such as organo chlorine based pesticides, heavy metals, pigments and dyes (Saraswathy and Balakumar, 2009) and must be treated before discharged into environment because of their recalcitrant nature and potential toxicity to animals and human (Levine *et al.*, 1991; Hildenbrand *et al.*, 1999; Martins *et al.*, 2002). Dyes also obstruct light penetration and oxygen transfer that affects water bodies (Franciscon *et al.*, 2009).

In recent years, numerous studies were carried out for the decolorization of textile effluent, including various physicochemical methods such as filtration, coagulation, chemical flocculation, use of activated carbon, advanced oxidation processes, ion exchange,

electrochemical and membrane process. Few of them are effective but with high cost, low efficiency and lack of selectivity of the process (Maier *et al.*, 2004; Kurniawan *et al.*, 2006).

Biological treatment offers a cheaper and environment friendly alternative to dye decolorization and wastewater reutilization in industrial process (Santos *et al.*, 2007; Mondal *et al.*, 2009). The general approach for bioremediation of textile effluent is to improve the natural degradation capacity of the indigenous microorganism that allows degradation and mineralization of dyes with a low environmental impact and without using potentially toxic chemical substances, under mild pH and temperature conditions (Chen *et al.*, 2003; Moosvi *et al.*, 2005; Pandey *et al.*, 2007; Kapdan *et al.*, 2007; Dhanve *et al.*, 2008; Khalid *et al.*, 2008). In addition, growth and decolorization ability are often a two stage process (Dhanve *et al.*, 2008; Khalid *et al.*, 2008). The present study was focused on the physicochemical characterization of textile effluent and screening of indigenous bacterial strains, isolated from dye contaminated sites, which had the potential not only to decolorize the dyes but also to achieve a good degree of mineralization and low toxicity at low running and maintenance cost.

Materials and Methods

Dyes and Chemicals

Dyes used in this study (i.e. Black WNN, Blue FNR, Blue RC, Red FN2BL, Diresul RDT black and TURQ blue) were obtained from Abhishek Industries Ltd., Barnala (India) and Nahar Oswal Denim, Mohali (India). Absorbance maxima (λ_{\max}) for each dye were obtained by scanning dye solution over visible range of 390-750nm using UV-Vis Spectrophotometer (Spectronix ST-2800, India) (Table 1). Media and chemicals were of analytical grade and procured from Himedia laboratories, Mumbai (India) and SD fine

Chem Ltd., Mumbai (India) respectively. The composition of the mineral salt media (MSM) and screening media (Mabrouk *et al.*, 2008) used in the present study was as follows: Glucose: 3g/l; (NH₄)₂SO₄: 2g/l; KH₂PO₄: 1g/l; K₂HPO₄: 10g/l; MgSO₄·7H₂O: 0.1g/l and NaCl: 5g/l.

TABLE 1 WAVELENGTH MAXIMA SCAN FOR VARIOUS DYE SAMPLES

| Dye | Type of dye | Maximum wavelength (nm) |
|-------------------|--------------|-------------------------|
| Black WNN | Reactive dye | 597 |
| Blue FNR | Reactive dye | 610 |
| Blue RC | Reactive dye | 592 |
| Red FN2BL | Reactive dye | 526 |
| TURQ blue | Reactive dye | 661 |
| Diresul RDT black | Vat dye | 630 |

Physicochemical Characterization of Textile Effluent

Effluent samples were collected in pre-sterilized polypropylene bottles from textile industries periodically and conventional parameters such as pH, TSS, TDS, Temp., EC, COD and BOD₅ were characterized as per the procedure recommended by standard method for the examination of water and wastewater (Clesceri *et al.*, 2005). Samples were stored at 4°C for the isolation of microorganisms.

Isolation of Bacteria from Soil and Wastewater Samples

Microbial isolations were carried out by serially diluting textile effluent and soil samples in sterile distilled water were subsequently plated onto nutrient agar plates (Cappuccino *et al.*, 1996). The plates were incubated at 37±2°C for 24 h and colonies with distinct morphology were picked up and purified by regular subculturing. The strains were maintained on slants of nutrient agar.

Initial Screening of Dye Decolorizing Bacterial Isolates Using Microtiter Plate Technique

Bacterial isolates were initially screened by microtiter plate technique (Lucas *et al.*, 2008) using selected dyes. Isolated cultures were cultivated in nutrient broth for 24 h before screening was done in mineral salt media (MS media). For initial screening, 10% (v/v) aliquot of each isolated strain (in nutrient broth) was inoculated into a 96 well microtiter plate, each containing 200 µL individual dye solution. Decolorization of the dye solution checked visually after 48 h incubation at 30±2°C. Strains that showed higher decolorizing potential were selected for further experimentation.

Final Screening and Dye Decolorizing Efficiency of Bacterial Isolates

Final screening was carried out using selected dyes in MS media (broth). Each selected strain was cultivated for 24 h in nutrient broth. A 5% (v/v) of the inoculum was then transferred into 250 ml Erlenmeyer flasks containing 50 ml of MS media. A final concentration of 100 mg/l of dye was added into each flask and absorbance was taken at their absorbance maxima (λ_{max}) initially (t₀) after a period of 24 h (t₂₄) and /or up to 72 h (t₇₂) at 30±2°C under static (anoxic) and shaking (aerobic) conditions (150 rpm). Based on the reduction in absorbance, the percentage of decolorization was estimated. Strains that exhibited a high potential of decolorizing ability were chosen for further experimentation.

Decolorization Assay

Decolorization activity expressed in terms of percentage was determined. The decrease in absorbance was monitored at λ_{max} for particular dye. Decolorized sample (5 ml) withdrawn periodically, was centrifuged at 10000 rpm for 15 mins and its absorbance was measured at λ_{max} of the dye. The uninoculated dye free medium was used as blank. All assays were performed in triplicate and compared with uninoculated control. The color removal efficiency of bacterial isolates was expressed as the following equation (Chen *et al.*, 2003; Ali *et al.*, 2009).

$$\text{Decolorization (\%)} = [I - F] \times 100 / I \quad (1)$$

Where, I = initial absorbance; F = final absorbance of decolorized medium.

Statistical Analysis

The experiments were performed repeatedly and the samples were analyzed in the replicates of three. The results obtained from each set of data have been expressed in terms of mean (average) and standard error by using Microsoft Excel (version Windows 2007).

Results and Discussion

Characterization of Textile Effluent

Textile effluent samples collected from Abhishek Industries Ltd., Barnala (India) and Nahar Oswal Denim, Mohali (India) were reddish brown and black in color with pungent smell. It was found that pH of untreated effluent depends upon the types of process being used in particular industry. Generally, processes in the industries were carried out at alkaline pH, and

it was observed that variations in pH of untreated effluent ranged from 10.5-11.5 and COD: 121-760 mg/l, BOD₅: 56-120 mg/l, whereas the treated effluent had the pH range from 7.5-8.5, COD: 40-246 mg/l and BOD₅: 15-30 mg/l. TDS and TSS in effluent were 548-87 mg/l and 90-20 mg/l (Table 2) and reduced to half after treatment, which was within the permissible limit and did not cause any harmful effect if released into the fields and natural water resources. The color absorbance of the treated sample was found to be higher than that of control, which was due to the presence of dye content. The pollutant load of the effluent which had adverse effects on aquatic flora, fauna and even human beings, was found to be higher than the permissible limit. Hence decolorization and degradation of dye present in textile effluent were needed. The values of BOD and COD were less in the treated sample in comparison to the very high values of BOD and COD in effluent (Ali *et al.*, 2009; Saraswathy *et al.*, 2009).

TABLE 2 PHYSICOCHEMICAL CHARACTERIZATION OF TEXTILE EFFLUENT COLLECTED FROM DIFFERENT TEXTILE INDUSTRIES

| Parameters | Industry 1* | | Industry 2* | |
|-------------------------|------------------|----------------|------------------|----------------|
| | Untreated Sample | Treated Sample | Untreated sample | Treated sample |
| pH | 11.5±0.50 | 7.5±0.5 | 10.5±1.5 | 8.5±0.5 |
| Temperature (°C) | 57.6±2.51 | 38±1 | 38±1.73 | 38±2 |
| Electrical conductivity | 924±1 | 1720±2 | 394±2 | 1250±2 |
| TSS (mg/l) | 90±1 | 19±2 | 20±2 | 13±2 |
| TDS (mg/l) | 548±1 | 202±2.64 | 87±1 | 27±1 |
| COD (mg/l) | 760±2 | 246±2 | 121±2 | 40±1 |
| BOD ₅ (mg/l) | 120±1 | 30±1 | 56±2.64 | 15±1 |
| Color (OD at 600 nm) | 0.230±0.01 | 0.124±0.001 | 1.640±0.02 | 1.140±0.04 |
| Color (Pt Co) | 3500±2 | 300±4 | 300±0.04 | 450±2 |

*Industry 1 Abhishek Industries Ltd. Barnala, Punjab (India)

Industry 2 Nahar Oswal Denim, Mohali, Punjab (India)

Isolation of Bacterial Isolates from Textile Effluent, Sludge and Soil Samples

Several bacterial colonies of distinct morphology and color were observed on nutrient agar plates after

incubation at 37°C for 24 h. Forty five bacterial cultures isolated from soil and effluent samples collected from Nahar Oswal Denim, Mohali were named NF-1 to NF-33, three isolates from effluent sample collected from Abhishek Industries Ltd., Barnala, named TI-I to TI-III and nine isolates obtained from soil samples contaminated with dye wastewater from field near local dyers, were named TrS1-TrS9. Numerous researchers isolated efficient dye decolorizing bacteria from the textile dye effluent, (Ali *et al.*, 2003; Kaushik and Malik, 2009; Khadijah *et al.*, 2009; Prasad and Rao, 2010; Ponraj *et al.*, 2011) activated sludge (Chen *et al.*, 2009; Vasileva *et al.*, 2009; Wang *et al.*, 2009) and soil contaminated with dye (Leena and Selvaraj, 2008; Mabrouk and Yusef, 2008; Telke *et al.*, 2008) collected from the waste disposal sites, lake-mud and wastewater treatment plant which indicated the natural adaptation of these isolates to high dye concentration (Khehra *et al.*, 2005) and their survival in the presence of toxic dyes (Abd El-Rahim *et al.*, 2003).

Initial Screening of Dye Decolorizing Bacterial Isolates Using Microtiter Plate Technique

Lucas *et al.*, (2008) developed a microtiter plate based method for the fast screening of numerous fungal strains for their ability to decolorize textile dyes individually or by mixture of dyes. Bacterial isolates were initially screened with different dyes using microtiter plate technique (Plate 1) and screening of bacterial isolates showing positive response to decolorization of different dyes as shown in Table 3.

TABLE 3 INITIAL SCREENING OF BACTERIAL ISOLATES FOR DECOLORIZATION OF DIFFERENT DYES BY MICROTITRE PLATE TECHNIQUE

| Name of Dyes | No. of screened bacteria isolates | Screened Bacterial Isolates |
|-------------------|-----------------------------------|--|
| Black WNN | 17 | TrS2, TrS3b, TrS5, TrS7, TrS8 NF-4, NF-6, NF-10, NF-13, NF-14, NF-15, NF-20, NF-22, NF-23, NF-30, NF-32, TI-II |
| Blue FNR | 12 | TrS2, TrS3, TrS4, TrS5, TrS6, TrS7, NF-8, NF-13, NF-15, NF-22, NF-23, NF-33 |
| Diresul RDT Black | 10 | NF-3, NF-6, NF-7, NF-12, NF-14, NF-15, NF-20, NF-21, NF-22, NF-23 |
| Red FN2BL | 1 | NF-23 |
| TURQ Blue | 1 | NF-23 |

It was observed that Black WNN was decolorized by bacterial isolates viz. TrS2, TrS3b TrS5, TrS7, TrS8, NF-4, NF-6, NF-10, NF-13, NF-14, NF-15, NF-20, NF-22, NF-23, NF-30 and NF-32, TI-II, similarly, Blue FNR was decolorized by bacterial isolates such as TrS2, TrS3,

TrS4, TrS5, TrS6, TrS7, NF-8, NF-13, NF-15, NF-22, NF-23 and NF-33, whereas, Blue RC was exclusive from being decolorized by these bacterial isolates. Diresul RDT black was decolorized by bacterial isolates NF-3, NF-6, NF-7, NF-12, NF-14, NF-15, NF-20, NF-21, NF-22 and NF-23. Red FN2BL and TURQ blue were decolorized only by NF-23.

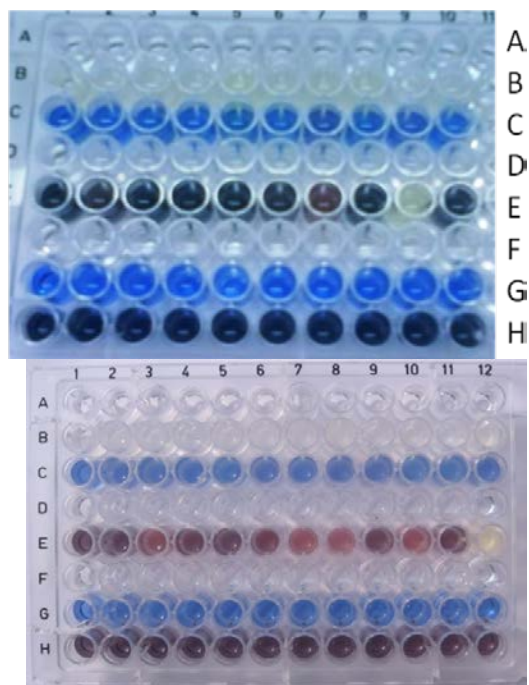


PLATE 1 MICROTITER PLATE CONTAINING BLUE RC AND BLACK WNN DYE, INOCULATED WITH ISOLATED BACTERIAL ISOLATES AFTER 48 H INCUBATION.

WELLS LANES: - A: WATER CONTAINING WELLS; G AND H: UNINOCULATED WELLS; C AND E: INOCULATED WELLS.

WELL COLUMN: - 9 AND 12: COMPLETE DECOLORIZATION OF BLACK WNN DYE

Final Screening and Dye Decolorizing Efficiency of Bacterial Isolates

Decolorization ability of initially screened seventeen isolates was studied in shake flask for the decolorization of Black WNN dye (100 mg/l) and 95% decolorization was shown by NF-23 followed by NF-14 (60.1%) > NF-22 (58.2%) > NF-20 (56.8%) > NF-30 (54.4%) (Fig. 1) under static condition, whereas all these isolates under shaking condition have shown only 6-19% decolorization. Similarly, TrS7 showed 62.7% decolorization of Black WNN dye followed by TrS2 and TrS5 i.e. 60.8% and 58.3% respectively (Fig. 2) and showed 6-25% decolorization under shaking condition after 72 h of incubation, whereas only 12% and 3% decolorization were observed with bacterial isolate TI-II under static and shaking condition respectively. Dye removal in static condition,

suggested that dye removal by these cultures was an anaerobic process favored by Chen *et al.*, (2003) that displayed 80% decolorization by *Aeromonas hydrophila* within 48 h under anoxic or anaerobic condition, however good growth in aerobic or agitated condition. The result was favored with the 96% decolorization of Reactive Red 180 in anaerobic condition, whereas only 13% in aerobic condition (Wang *et al.*, 2009) at 150 rpm accorded with the conclusion that none of these decolorizing bacteria was able to decolorize dyes under shaking (aerobic) condition (Khehra *et al.*, 2005; Moosvi *et al.*, 2005) as oxygen being a preferable terminal electron acceptor over the azo groups inhibited the anaerobic decolorization. These results suggested that NF-23, a type of facultative anaerobe, showed that oxygen was favorable to the growth of bacterium, though, it inhibited the yield of degradation related enzyme.

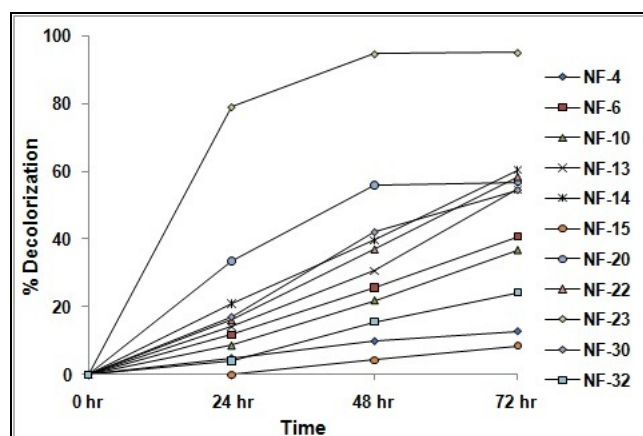


FIG. 1 DECOLORIZATION OF BLACK WNN DYE BY BACTERIAL ISOLATES (NF-4, NF-6, NF-10, NF-13, NF-14, NF-15, NF-20, NF-22, NF-23, NF-30 AND NF-32) UNDER STATIC CONDITION

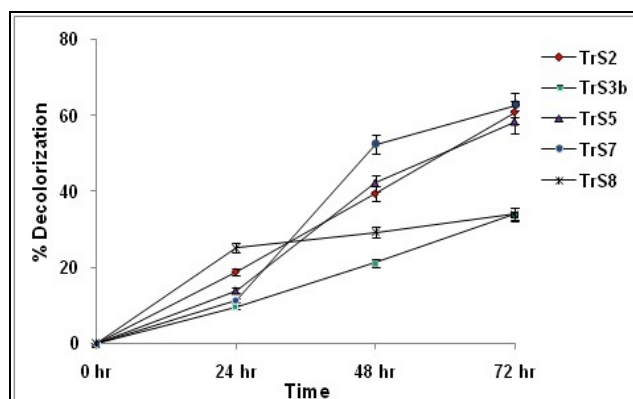


FIG. 2 DECOLORIZATION OF BLACK WNN DYE BY BACTERIAL ISOLATES (TrS2, TrS3b, TrS5, TrS7 AND TrS8) UNDER STATIC CONDITION

Further, decolorization of Diresul RDT Black (100 mg/l) was shown by eight bacterial isolate under static and shaking condition. Maximum decolorization of Diresul RDT black under static condition was shown by NF-21

(91.4%) followed by NF-7 (87.0%) > NF-15 (84.7%) > NF-6 (84.2%) > NF-12 (81.0%) > NF-14 (78.0%) > NF-23 (72.2%) > NF-20 (69.4%) and NF-3 (62.6%), respectively (Fig. 3) but in shaking condition decolorization reduced to 78.1 > 78.0 > 67.1 > 63.7 > 63.1 > 62.6 > 50.8 > 46.3 > 38.0% by NF-21, NF-15, NF-20, NF-7, NF-6, NF-23, NF-14, NF-12 and NF-23 respectively after 72 h of incubation. All these isolates were able to decolorize Diresul RDT Black dye under aeration considerably.

Bacterial isolates TrS6, TrS7 (Fig. 4) NF-23 and NF-33 (Fig. 5) decolorized 69.5, 59, 64.6 and 48% of Blue FNR dye (100 mg/l) respectively. Similarly, 80% decolorization of Red FN2BL dye (100 mg/l) was observed with bacterial isolate NF-23, whereas only 32% decolorization was obtained for TURQ Blue (50 mg/l) (Fig. 6) after 72 h of incubation. During final screening of sixteen bacterial isolates, NF-23 (Black WNN, Diresul RDT black, Blue FNR and Red FN2BL), NF-22 (Black WNN and Diresul RDT black) and NF-21 (Diresul RDT black), efficiently decolorized the dyes within 24-72 h. These isolates were further, morphologically and biochemically characterized (Table 4). Kaushik *et al.*, (2009) reported that decolorization for various dyes under shaking condition after 50 h incubation was: Acid Sulphone blue (82.2%), Acid Navy Blue (75.8%), Fast Red (46.4%) as compared to 48.5, 68.9 and 40.8% in static condition by bacterial isolate CBE, suggesting that dye decolorization was an aerobic process.

Various researchers had isolated and studied different dye decolorizing bacterial isolates using different types of dye. A bacterial strain AZO29 isolated from activated sludge showed 100% decolorization of Amaranth (azo) dye (Vasileva *et al.*, 2009) in an anoxic batch reactor after 72 h of incubation at the presence of 1400 mg/l of dye. Several facultative anaerobic bacterial strains including *Sphingomonas* sp. (Kudlich *et al.*, 1997), *Pseudomonas luteola* (Hseuh and Chen, 2003), *Streptococcus faecalis* and *Klebsiella pneumoniae* (Wong *et al.*, 1996) had described as being capable of reducing azo dyes. Similarly, *Aeromonas hydrophila*, *Proteus vulgaris* and *Providencia rettgeri* (azo dye decolorizing bacteria) isolated from leather industry wastewater showed 95, 94.5 and 94.5% decolorization against Acid black 24 under static conditions within 336 h, whereas under shaking condition decolorization reduced to 74, 61 and 90% by *Proteus vulgaris*, *Providencia rettgeri* and *Aeromonas hydrophila* respectively (Ozdemir *et al.*, 2006).

Telke *et al.*, (2008) reported 90% decolorization under static condition within 48 h. In addition, Khadijah *et al.*, (2009) screened nine bacterial isolates based on their

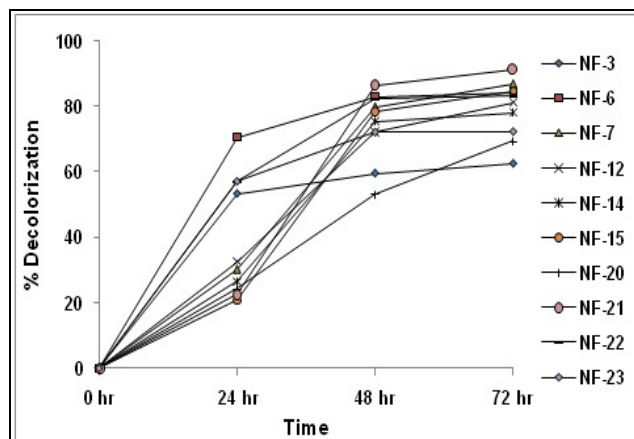


FIG 3 DECOLORIZATION OF DIRESUL RDT BLACK DYE BY BACTERIAL ISOLATES (NF-3, NF-6, NF-7, NF-12, NF-14, NF-15, NF-20, NF-21, NF-22 AND NF-23) UNDER STATIC CONDITIONS

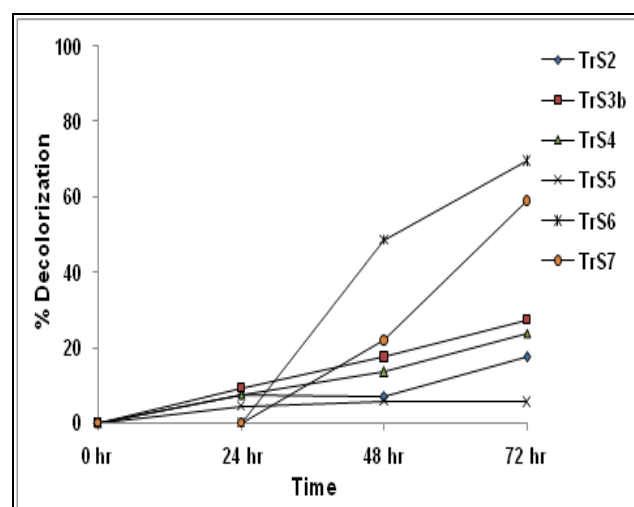


FIG. 4 DECOLORIZATION OF BLUE FNR DYE BY BACTERIAL ISOLATES (TRS2, TRS3b, TRS4, TRS5, TRS6, AND TRS7) UNDER STATIC CONDITIONS

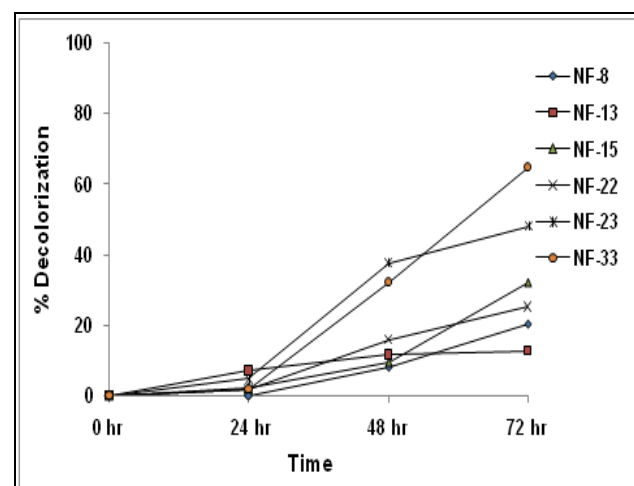


FIG. 5 DECOLORIZATION OF BLUE FNR DYE BY BACTERIAL ISOLATES (NF-8, NF-13, NF-15, NF-22, NF-23 AND NF-33) UNDER STATIC CONDITIONS

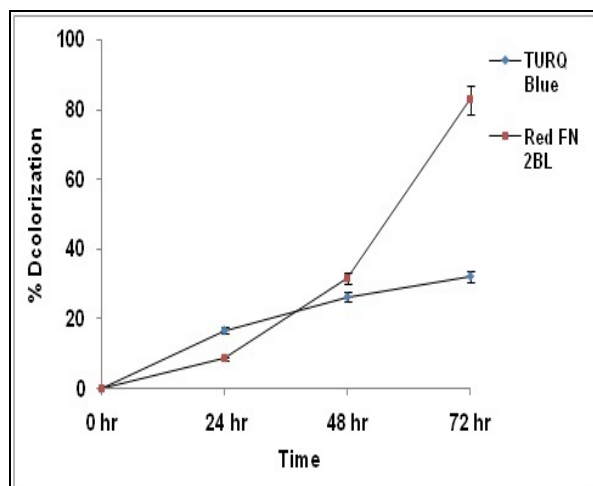


FIG. 6 DECOLORIZATION OF TURQ BLUE AND RED FN2BL DYE BY BACTERIAL ISOLATE NF-23 UNDER STATIC CONDITIONS

TABLE 4 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF ISOLATES NF-21, NF-22 AND NF-23

| Characteristics | NF-21 | NF-22 | NF-23 |
|-----------------------------|-------|-------|-------|
| Gram staining | + | - | + |
| Spore staining | - | - | + |
| Starch hydrolysis | + | + | + |
| Casein hydrolysis | - | + | - |
| Citrate utilization | - | - | - |
| Gelatin liquifaction | + | - | - |
| H ₂ S production | - | - | - |
| MR | + | + | + |
| Nitrate reduction | + | + | + |
| Indole | - | - | + |
| Catalase | + | + | + |
| Oxidase | - | - | + |
| Urea | + | - | - |
| Acid production from | | | |
| Arabinose | - | + | - |
| Galactose | + | + | - |
| Glucose | + | + | + |
| Mannitol | - | - | - |
| Raffinose | - | - | - |
| Xylose | - | + | - |
| Sucrose | + | - | - |
| Fructose | + | + | - |
| Growth at 12°C | - | + | - |
| Growth at 25°C | + | + | + |
| Growth at 37°C | + | + | + |
| Growth at 42°C | + | + | + |
| Growth at pH 5.2 | + | + | - |
| Growth at pH 8.0 | + | + | - |
| Growth at pH 9.0 | + | + | - |
| Growth at pH 10.5 | + | + | - |
| Growth on NaCl 2% | + | + | + |
| Growth on NaCl 5% | + | + | - |
| Growth on NaCl 7% | + | - | - |
| Growth on NaCl 10% | + | - | - |

abilities to decolorize a wide spectrum of dyes from their 1540 isolates and further developed microbial consortia, which had shown 70-100% decolorization. Ponraj *et al.*, (2011) utilized *Bacillus* sp., *Klebsiella* sp.

Salmonella sp. and *Pseudomonas* sp. isolated from textile dye effluent and found 89% of decolorization of Orange 3R dye by *Bacillus* and *Pseudomonas* sp., followed by *Salmonella* sp. (80%) and *Klebsiella* sp. (76%) after 144 h of incubation. The reported results by various researchers favored the anaerobic decolorization of azo reactive dyes by bacterial isolates such as NF-23, NF-22, NF-14, NF-15, NF-21, NF-20, NF-33, TrS2, TrS6 and TrS7. Due to efficient decolorization capability, these isolates could be used to develop microbial consortia for the decolorization and complete mineralization of dyes from textile effluent as the biological treatment of textile effluent not only decolorized the effluent but also reduced the cost of effluent treatment process. Finally screened bacterial isolates able to decolorize different dyes were summarized in (Table 5).

TABLE 5 FINAL SCREENING OF BACTERIAL ISOLATES FOR THE DECOLORIZATION OF DYE SAMPLES

| Name of Dye | No. of screened bacteria | Name of screened Bacteria |
|-------------------|--------------------------|---|
| Black WNN | 9 | TrS2, TrS5, TrS7 NF-13, NF-14, NF-20, NF-22, NF-23, NF-30 |
| Blue FNR | 4 | TrS6, TrS7, NF-23, NF-33, |
| Diresul RDT Black | 8 | NF-6, NF-7, NF-12, NF-14, NF-15, NF-21, NF-22, NF-23 |
| Red FN2BL | 1 | NF-23 |

Conclusion

Except Blue RC, all five dyes were completely decolorized by bacterial isolate NF-23. Bacterial isolates NF-23, NF-22 and NF-21 were selected for the decolorization studies based on their higher potentials to decolorize Black WNN dye (Reactive Black 5). Decolorization by NF-23 was found to be 95% within 24 h, whereas NF-22 and NF-21 exhibited the rate of decolorization of about 89% and 91% respectively during final screening. Hence, these isolates can be used for the treatment of textile effluent in the form of microbial consortia. The prospect plan of work is focused on optimizing process parameters to attain maximum decolorization for different dyes and to study the decolorization potential of screened bacterial isolates individually or by developing consortia.

ACKNOWLEDGMENT

This work was funded by the University Grants Commission, New Delhi as research project with

reference no. 35-28/2008(SR). The author(s) are grateful to Head of the Institute for providing the laboratory facility to carry out the research work.

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